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THE MYOLOGY OF THE HEAD AND NECK OF THE INDIAN TORTOISE, *TESTUDO ELEGANS*

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I N our earlier paper (1954) we have described the musculature of the head and neck of the pond turtle, *Lissemys punctata*. This paper deals with that of the tortoise, *Testudo elegans* which possesses a head and neck with restricted movements in contrast to those of the former.

Description of the Muscles

The *adductor mandibulæ* in the lower tertrapods consists of three muscle masses, acting as the adductor of the lower jaw, the *adductor mandibulæ externus*, *adductor mandibulæ medius* and the *adductor mandibulæ internus*. In testudo, the *adductor mandibulæ externus* (Figs. 1, 2, 3, and 4) is a powerful muscle concerned with the movements of the lower jaw. The muscle has a wide-spread origin from the inner surface of the squamosal, the ventral as well as the lateral surface of the supraoccipital crest and also from the dorso-lateral part of the parietal. The fibres arising from the squamosal decussate with those from the
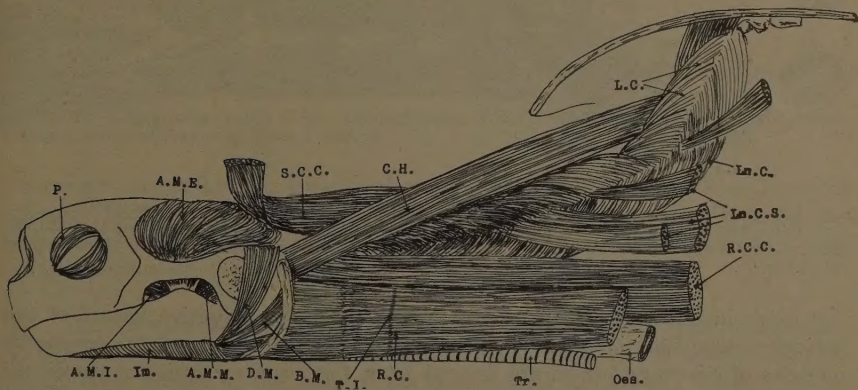
FIG. 1. Lateral view of the head and neck of Testudo with the constrictor colli and the rectus capitis cervico-plastralis removed and the spinalis cervico-capitis reflected.
A.M.E., Adductor mandibulæ externus ; A.M.I., Adductor mandibulæ internus ; A.M.M., Adductor mandibulæ medius ; B.M., Branchiomandibularis ; C.H., Constrictor hyoideus ; D.M., Depressor mandibulæ ; Im., Intermandibularis ; L.C., Longissimus cervicis ; Ln. C., Longus colli ; Ln.C.S., Muscles of the Longus colli series in the trunk region ; Oes., Oesophagus ; P., Palpebralis ; R.C., Rectus cervicis ; R.C.C., Retrachens capitis collique ; S.C.C., Spinalis cervico-capitis ; T.I., Tendinous intersection ; Tr., Trachea.

supraoccipital and parietal and finally become one single muscle mass which turns downwards through the vacuity formed by the emargination of bones, to get inserted on the coronoid process of the mandible. The vacuity in the skull is bounded on the lateral side by a transverse bony bar, the juguloquadrate bar, consisting of the jugal in front, the quadrate behind and the quadrato-jugal in the middle. This bony bar which is analogous to the zygomatic arch of mammals is also present in Lissemys and Trionyx. The *adductor mandibulæ externus* in Testudo passes behind the jugulo-quadrate bar, while in Lissemys it is split at the level of this bony bar into two muscles, a lower and outer, the *masseter*, arising from the jugulo-quadrate bar and inserted on the lower edge of the mandible, and the upper and the inner part, the *temporalis*, arising from the usual place of origin of the *adductor mandibulæ externus* as seen in Testudo, to be inserted on the coronoid process of the mandible. Thus the *temporalis* and the *masseter* muscles in Lissemys are derived from the primitive *adductor mandibulæ-externus* (George and Shah, 1954). In the mammals too the zygomatic arch effects the formation of the two muscles, the *temporalis* and the *masseter* from the *adductor mandibulæ externus* (Quiring 1950). Inspite of the fact that the jugulo-quadrate
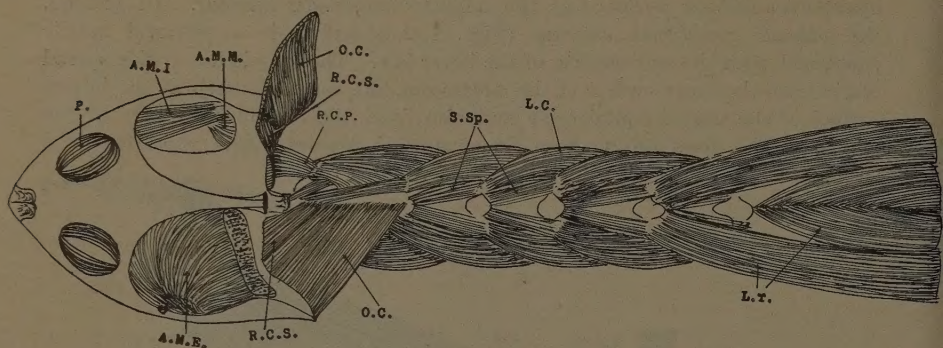
Fig. 2. Dorsal view of the head and neck of Testudo showing the epaxial muscles.
L.T., The Longissimus muscles arising from the carapace ; O.C., Obliquus capitis ; R.C.S., Rectus capitis superficialis ; R.P., Rectus profundus ; S.Sp., Semispinalis. (Other abbreviations as in Fig. 1).

bar exists in the Testudo and Emys (Owen, 1866), the primitive nature of the *adductor mandibulæ externus* is still retained. The *masseter* and the *temporalis* muscles of Lissemys (which are derived from the primitive *adductor mandibulæ externus*) as well as the *adductor mandibulæ externus* of Testudo are innervated by a branch of the Trigeminal nerve.

The *adductor mandibulæ medius* (Figs. 1 and 2) arises from the ventro-lateral part of the parietal, posterior part of the pro-otic and also from the inner anterior border of the quadrate. All the fibres converge and form a tendinous part which gets inserted on the coronoid process of the mandible.

The *adductor mandibulæ internus* (figs. 1 and 2) is similar to that of Lissemys. It has a wide range of origin namely from the anterior inner border of the jugal, posterior border of the maxilla, outer border of posterior end of the palatine and the anterior outer border of the pterygoid. It runs backwards and downwards towards the articular bone of the mandible where it gets inserted by a thin tendon.

The *depressor mandibulæ* (Figs. 1, 3 and 4) which acts as a depressor of the lower jaw arises from the outer surface of the squasmosal and proceeding down-
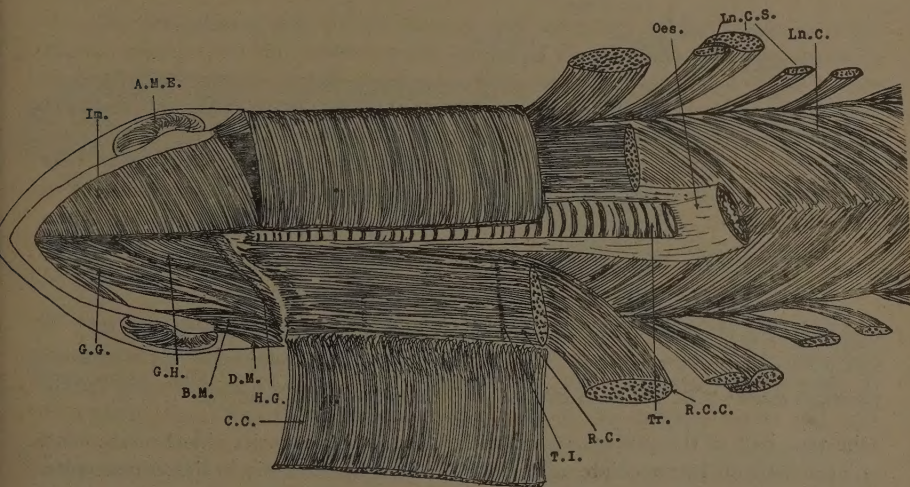
Fig. 3. Ventral view of the head and neck of Testudo with intermandibularis muscle removed on one side and the constrictor colli of the same side reflected.

C.C., Constrictor colli; G.G., Genioglossus; G.H., Geniohyoideus; H.G., Hypoglossus. (Other abbreviations as in Figs. 1 and 2).

wards gets inserted on the lower border of the angular. This muscle in Lissemys (George and Shah 1954) unlike in Testudo consists of two parts an anterior and a posterior one. The fibres of both the parts decussate and run downwards to gain their insertion on the mandible.

The *intermandibularis* (Figs. 1 and 3) which is also similar to that in Lissemys, is the most superficial muscle on the ventral side of the head and extends from the entire lower border of one side of the mandible to the other. It has a median aponeurotic raphæ which is attached posteriorly to the hyoid. On contraction of this muscle the floor of the buccal cavity is lowered, and raised on relaxation.

The *constrictor colli* (Fig. 3) which forms a superficial sheath of muscle on the lateral and ventral sides of the neck arises in two parts. An anterior one

originates on each side, from a median aponeurotic raphæ on the mid-dorsal line of the neck at the level of the atlas and axis. The more extensive posterior part takes its origin from the lateral side of neural arches of the second to the sixth cervical vertebræ. The fibres of both the parts run laterally downwards and form a continuous flat muscular sheath over the lateral and ventral sides of the neck. Thereafter the fibres on one side of the anterior part and those of the anterior half of the posterior part of the same side meet the corresponding ones of the opposite side in the mid-ventral line to form a thin tendinous raphæ. The fibres of
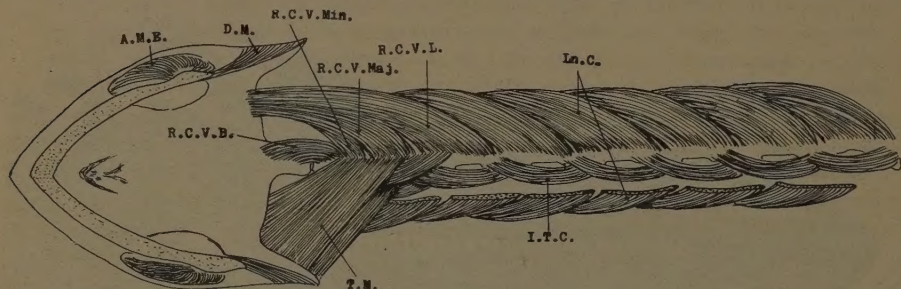

Fig. 4. Ventral view of the head and neck of Testudo showing the hypaxial muscles. The superficial muscles are cut on the right side.
I.T.C., Inter-transversarii colli ; R.C.V.B., Rectus capitis ventralis brevis ; R.C.V.L., Rectus capitis ventralis longus ; R.C.V.Maj., Rectus capitis ventralis major ; R.C.V.Min., Rectus capitis ventralis minor ; T.M., Trachelomastoideus. (Other abbreviations as in Figs. 1, 2, 3).

the rear half of the posterior part with those of the opposite side become continuous with an intermediate aponeurosis. This aponeurosis in the median plane on the ventral side is in continuation with the fibres of the anterior half of the muscle. In Lissemys (George and Shah, 1954), however, this muscle consists of only one part which arises from a fairly broad, membranous aponeurosis on the dorsum of the neck with the fibres of the anterior half running laterally downwards and turning to the ventral side of the neck to meet those of the opposite side through a common median raphæ, while the fibres of the posterior half converge towards the posterior border of the raphæ on the ventral side. A few posterior fibres that fail to reach the raphæ become aponeurotic and fuse with the aponeurosis formed by those of the opposite side. So in Testudo as in Emys (Owen, 1866) the *constrictor colli* forms a more or less complete muscular envelope of the whole of the neck, whereas in Lissemys it is restricted only to the anterior half of the neck. This difference in Lissemys is evidently due to the longer neck whose versatile movements would otherwise be restricted if this muscle did cover the entire neck as in the other two.

The *branchio-mandibularis* is similar to that in Lissemys as it(Figs. 1 and 3) arises from the distal end of the anterior cornu and running anteriorly gets inserted on the lower border of the angular and the posterior part of the dentary.

The *geniohyoideus* (Fig. 3). As in Lissemys this muscle arises from the inner border of the mandibular symphysis and after running posteriorly some of the fibres decussate with those of the other side in the mid-ventral line till the level of the basihyal. After this it runs obliquely backwards and outwards and finally gets inserted on the ceratohyal and the anterior border of the proximal end of the anterior cornu of its own side. As in Lissemys it brings about the movements of the tongue and the hyoid.

The *genioglossus* (Fig. 3), an elevator and protractor of the hyoid is a single muscle arising from the anterior lower border of the dentary and gains insertion on the lateral process of the basihyal. In Lissemys (George and Shah, 1954), however, it consists of two distinct muscles, the *genioglossus externus* and *genioglossus internus*, the former having a similar origin, course and insertion as that of the single *genioglossus* of Testudo, but the latter, though has the same origin, has its insertion on the anterior border of the proximal end of the anterior cornu. These two muscles in Lissemys, must have been derived from a composite *genioglossus* muscle probably to facilitate the greater throat movements seen in this animal.

The *hypoglossus* (Fig. 3) arises from the ventro-lateral and ventro-medial borders of the distal end of the anterior cornu and the fibres get inserted on the lateral process of the ceratohyal in Lissemys (George and Shah, 1954), while in Testudo it arises from the entire anterior border of the anterior cornu and gets inserted on the body of the ceratohyal. The muscle acts as a depressor and retractor of the hyoid even though its origin and insertion are different in the two animals.

The *hypoglosso-glossus* and the *hypoglosso-hyoideus* are the muscles concerned with the movements of the tongue and they are found only in the Chelonia and not in any other group of reptiles. They arise from a cartilaginous plate, the hypoglossum, situated under the tongue. The hypoglossum is a unique structure found only in the Chelonia, which in Testudo is smaller than the one found in Lissemys. The *hypoglosso-glossus* arises from the lateral margin of the hypoglossum and is inserted on the hyoid. The *hypoglosso-hyoideus* arises from the dorsal median surface of the hypoglossum and is inserted on the hyoid. We had ventured to name these two muscles as such (George and Shah, 1954), since we had not come across any mention of such muscles anywhere.

The *intercornuatus* which is responsible for the intercornual movements is a thin membranous muscle connecting the two cornua. Its fibres run somewhat obliquely outwards from the posterior border of the anterior cornu to the anterior border of the posterior cornu. As the posterior cornu in Testudo is very small compared to that of the Lissemys, this muscle is here very diminutive.

The *constrictor hyoideus* (Fig. 1) in Testudo compared to that of the Lissemys is a poorly developed muscle and arises on the lateral side, from the region of the neural spine of the seventh cervical vertebra and runs anteriorly downwards towards the hyoid to get inserted on the posterior border of the distal end of the anterior cornu. A few fibres of this muscle are also inserted on the posterior end of the squamosal. In Lissemys however (George and Shah, 1954) it arises as three fleshy fasciculi from the region of the neural spine of the fifth, sixth and the seventh cervical vertebræ. All the three fasciculi run anteriorly and after a short distance join to form a single muscle belly which runs anteriorly towards the hyoid. Again, this belly gets split up into three parts which are variously inserted. Such an elaborate modification of the muscle in Lissemys should be to bring about a powerful retraction of the head and neck, in addition to its primary function of being a depressor and an elevator of the floor of the buccal cavity and the hyoid. Since the neck in Testudo is comparatively short and the posterior cornu of the hyoid is not developed to the same extent as in Lissemys, the *constrictor hyoideus* remains as a single muscle.

The *tensor nasalis* is absent in Testudo and there is no snout as in Lissemys and Trionyx.

The *rectus capitis cervico-plastralis* arises from the sternal plate of the plastron by a fleshy origin and runs anteriorly into the neck on its lateral side as a cylindrical belly which on approaching the base of the skull turns to the dorsal side and finally gets inserted on the posterior end of the dorso-lateral part of the squamosal but in Lissemys (George and Shah, 1954) the muscle gets flattened out in the region of the squamosal and gets merged with the skin, which in turn gets closely attached to its place of insertion on the squamosal. When the muscles of both the sides contract simultaneously the head and neck are flexed and retracted, but when the muscle of only one side contracts, a lateral rotation of the head and lateral flexion of the neck are effected.

The *spinalis cervico-capitis* (Fig. 1) arises by five muscle bellies each one from the region of the neural spines of the second to the sixth cervical vertebræ respectively. All the parts run anteriorly and unite with one another to form one single muscle mass which goes forwards. Some fibres on the lateral side get inserted on the posterior half of the inner dorsal border of the squamosal, and the medial ones gain their insertion on the posterior end of the supraoccipital crest, while the fibres in the middle which form the major bulk of the muscle are inserted on the under surface of the skin at the basal region of the skull. The skin is firmly fixed on the parietals and the jugulo-quadrate bar. The parts of this muscle corresponding to the atlas, seventh and eighth cervical vertebræ are absent. This muscle in Lissemys (George and Shah, 1954) is formed of four parts instead of five as in Testudo. The presence of an additional part in Testudo is perhaps to support the neck which has undergone a severe bend at this position owing to the carapace having become dome-shaped. The muscle in both the animals acts as an elevator and lateral rotator of the head.

The *semispinalis* (Fig. 2) shows more or less a typically primitive segmental arrangement. It arises from the posterior zygapophysis of one vertebra and runs anteriorly to get inserted on the posterior border of the region of the neural arch of the next preceding vertebra. This arrangement is seen throughout the neck. In sharp contrast to this the *semispinalis* system in Lissemys has undergone great modification and exhibits considerable complexity of structure by linking up with the *longissimus* system (George and Shah, 1954).

The *longissimus cervicis* (Figs. 1 and 2) muscles also differ greatly from those of Lissemys. This muscle in Lissemys shows several divisions and subdivisions and to add to the complexity, some of them get even linked up with the *semispinalis* group and thereby produce a system of muscles for a characteristically varied, powerful and effective muscular action (George and Shah, 1954). In Testudo on the contrary this muscle shows basically the primitive segmental arrangement. The part corresponding to the eighth cervical vertebra arises from the lateral border of the neural arch of the same vertebra and the fibres join with a few of those of the *longissimus thoracis* (Fig. 2) belonging to the second trunk vertebra, to gain a common insertion on the region of the neural spine of the seventh cervical vertebra. The one corresponding to the seventh cervical vertebra arises from the lateral side of the neural arch of the same vertebra and joins with the fibres of the *longissimus thoracis* belonging to the first trunk vertebra to gain a common insertion at the region of the neural spine of the sixth cervical vertebra and also the anterior border of the neural arch of the same side of the same vertebra. The part belonging to the sixth vertebra arises fom the lateral side of the neural arch of the sixth vertebra and runs anteriorly forwards and inwards to get inserted at the region of the neural spine of the fifth and also on the anterior border of the neural arch of the same side of the same vertebra. This is a typical arrangement of origin, course and insertion of *longissimus cervicis* in Testudo. The parts corresponding to the fifth and fourth cervical vertebræ also show the above said arrangement in their origin, course and insertion, but those of the third and second cervical vertebræ do not get inserted at the usual place of insertion but join with each other and have a common insertion on the lateral process of the atlas. The *longissimus* of the first and second thoracic vertebræ are well-developed and they arise partly from the carapace and partly from the first thoracic vertebra which is somewhat free from the carapace. No fibres arise from the second thoracic vertebra because it is competely fused with the carapace. These parts of the muscle which are conspicuously powerful get inserted at the region of the neural spine of the sixth and seventh cervical vertebræ and at the anterior half of the same region of the eighth cervical vertebra.

The occipital muscles which serve as the lateral rotators of the head comprise a lateral and a medial part. The former consists of the *obliquus capitis* and the latter of the *rectus capitis*.

The *obliquus capitis* (Fig. 2) arises from the region of the neural spine on the axis and atlas vertebræ. The fibres run obliquely outwards and get inserted on the inner border of the squamosal and the pro-otic bones. In Lissemys this muscle formed of two bellies, an upper one having the same insertion as the *obliquus capitis* of Testudo and a lower one (absent in Testudo) gets inserted on the tendinous strip of the *longissimus* of the third cervical vertebra (George and Shah, 1954).

The *rectus capitis superficialis* (Fig. 2) arises from the region of the neural spine of the atlas and the fibres run obliquely forwards to be inserted on the pro-otic, opisthotic and the side of the basioccipital.

The *rectus capitis profundus* (Fig. 2) is a small muscle arising from the lateral process of the atlas and gets inserted on the pro-otic and the opisthotic bones.

The *rectus cervicis* (Figs. 1 and 3) in Testudo arises from the dorsal surface of the distal end of the coracoid, proceeds forwards through the trunk and runs ventrally through the entire length of the neck. The muscle in the beginning is somewhat cylindrical but later flattens out and after passing over the posterior cornu of the hyoid gets inserted on the posterior border of the anterior cornu. A few fibres, however, run over the anterior cornu and obtain their insertion on the basihyal and a few others get inserted on the proximal part of the posterior cornu. There is a thin tendinous intersection present on the muscle at a position slightly anterior to its middle. In Lissemys (George and Shah, 1954) this muscle is much more modified than in Testudo. Though it arises from the dorsal side of the distal end of the coracoid as in Testudo, the muscles of either side as they enter into the neck region from the trunk converge and the median fibres of both the muscles decussate at a place more or less in the middle of the neck and then proceed anteriorly as a single flat muscle. Shortly after this decussation, is present a thick broad tendinous intersection after which the muscles again separate and run forwards and laterally. After parting company, each one splits up into three parts which lie one over the other. The dorsal part runs forwards along the ventral side of the œsophagus and finally merges with the wall of the œsophagus and the pharynx. The middle part gets inserted on the hyoid where the ceratohyal and the basihyal meet while the ventral part runs over the posterior cornu and gets inserted on the anterior border of the ceratohyal by a thin tendon. Though the tendinous intersection in the muscle in Testudo is present, the muscle of one side neither meets the fellow of the opposite side nor splits into three different parts having different points of insertion. The three parts together of this muscle in Lissemys correspond to the entire muscle of Testudo. The mammalian *sternothyroid* and *omohyoid* are derived from the primitive *rectus cervicis* of the lower tetrapods. The ventral and middle parts of this muscle in Lissemys could be considered as homologous to the *omohyoid* and the *sternothyroid* respectively of the mammals, while the entire *rectus cervicis* of Testudo to both the above mammalian muscles. As pointed out before (George and Shah, 1954) the third (dorsal) part of this muscle in Lissemys does not correspond to any other muscle in the vertebrates.

The *retrachens capitis collique* (Figs. 1 and 3) arises very close to the vertebral column by four fleshy fasciculi from the neural plates covering the third to seventh trunk vertebræ.   Of the four fasciculi, the posterior one which is very massive arises from the neural plate covering the sixth trunk vertebra and also partly from the anterior half of the neural plate covering the seventh vertebra. The muscle runs anteriorly and enters the neck.   After traversing the entire length of the neck it gets inserted partly on the basioccipital by a thick tendon and partly on the inner posterior border of the squamosal.   The next two fasciculi arise from the side of the fifth and the fourth trunk vertebræ respectively, both running side by side anteriorly before entering the neck region.   The one which arises from the side of the fifth trunk vertebra and its corresponding neural plate gets inserted on the diapophysis of the fourth cervical vertebra and the other which arises from the side of the fourth trunk vertebra and its corresponding neural plate gets inserted on the diapophysis of the fifth cervical vertebra.   The anteriormost one arises from the body of the third trunk vertebra and the neural plate corresponding to the same vertebra.   It runs anteriorly and enters the neck to get inserted on the diapophysis of the sixth cervical vertebra.

In describing the *retrachens capitis collique* in *Emys europea*, Owen (1866) states that it arises by six fleshy fasciculi from the neural arches and spines of the dorsals and proceeds forwards blending together and gets detached into four tendinous insertions.   Here in Testudo there are four such fasciculi and they remain completely separate without any blending whatsoever.   On studying the nerve supply to these four fasciculi we find that the largest posteriormost one, which is inserted on the base of the skull is innervated by a branch from the middle cervical ganglion of the vagus anteriorly and the sixth thoracic nerve at the origin of the muscle, whereas the other three fasciculi, which are inserted on the diapophyses of the cervical vertebræ obtain their nerve supply from the third, fourth and fifth thoracic nerves.   The fact that these three fasciculi remain completely separate from the posteriormost and largest fasciculus and that each of them is innervated by altogether a different source of nerve supply show that they are totally separate muscles.   It is possible therefore that these three fasciculi correspond to the *longus colli* series of muscles and do not form a part of the *retrachens capitis collique*. The fact that the largest posteriormost fasciculus is innervated at its origin by the sixth thoracic nerve denòtes that this posterior part of the fasciculus is formed by one of the *longus colli* series of muscles corresponding to the sixth trunk vertebra. The presence of the three fasciculi arising from the third, fourth and fifth trunk vertebræ in Testudo and their absence in Lissemys (George and Shah, 1954) shows that they became necessary in order to support the neck which underwent a bend at this level of the body owing to the carapace acquiring the shape of a dome.

The *retrachens capitis collique* in Lissemys (George and Shah, 1954) is a highly specialised muscle different from that of Testudo in its origin, course and insertion. While describing this muscle in Lissemys, we (1954) had considered it as consisting

of two parts an anterior cervical and a posterior trunk one, the anterior part corresponding to the mammalian *sterno-cleidomastoid* muscle derived from the primitive *cucularis* and the trunk one derived from the primitive *pubo-ischio-femoralis* from which the *psoas* muscles of mammals were derived. A fuller discusion, however, on the *retrachens capitis collique* muscle in the region of the trunk in Lissemys and Testudo we reserve for a future discourse dealing with the myology of the trunk and tail.

The hypaxial muscles of the neck in Testudo consists of two layers but those in Lissemys (George and Shah, 1954) of three. The ventralmost layer of muscles in Testudo is the *longus colli*.

The *longus colli*, (Figs. 1 and 4) in Testudo, corresponding to the eighth cervical vertebra arises from the mid-ventral line of the body of the same vertebra and the fibres run obliquely forwards and inwards. On its way the muscle splits into two parts, one upper and the other lower. The former gets inserted on the body of the seventh vertebra, while the latter becomes tendinous and gains a common insertion with the muscle the *intertransversarii colli*, on the posterior zygapophysis of the second preceding vertebra. The origin and insertion are the same for the *longus colli* series corresponding to the fourth to seventh cervical vertebræ. The muscle arising from the third cervical vertebra occurs in two flat bellies. The lower one shows the usual place of origin of the entire *longus colli* but the upper part, the *rectus capitis ventralis longus*, (Fig. 4) runs anteriorly and gets inserted on the pro-otic bone by a tendon. The muscles arising from the axis and atlas vertebræ, the *rectus capitis ventralis major* (Fig. 4) and *rectus capitis ventralis minor* (Fig. 4) respectively, join with the fleshy fibres of the *rectus capitis ventralis longus*.

There is no notable difference between the *longus colli*, the *rectus capitis ventralis longus*, the *rectus capitis ventralis major* and the *rectus capitis ventralis minor* muscles of Testudo and Lissemys.

The *rectus capitis ventralis brevis* (Fig. 4) in both Testudo and Lissemys arises from the anterior border of the atlas and the distal end of the basioccipital. It runs anteriorly for a very short distance and gets inserted on the anterior border of the basioccipital.

All these muscles, the *rectus capitis ventralis longus*, the *rectus capitis ventralis major* and *minor* and the *rectus capitis ventralis brevis* correspond to the *longus capitis* of mammals.

The *intertransversarii colli* (Fig. 4) muscles lie immediately dorsal to the *longus colli*. In Testudo these muscles are segmental and show the following arrangement of origin and insertion for the muscles of all the cervical segments. The muscle

corresponding to the last (eighth) cervical vertebra arises from the mid-ventral line of the body of the vertebra and the fibres run anteriorly and converge to get inserted on the posterior zygapophysis of the seventh cervical vertebra. The muscles of the second to seventh cervical vertebræ show this typical arrangement. The muscle corresponding to the first cervical vertebra arises from the mid-ventral line of the body of the same vertebra and gets inserted on the basioccipital. These muscles in Testudo and Lissemys bring about inter-vertebral movements. The difference between the muscles of Testudo and Lissemys is only in their origin. In Lissemys they arise by two heads, one each from two adjoining vertebræ and in Testudo by only one head. This is necessitated by the presence in Lissemys of a long neck that performs a good deal of inter-vertebral movements unlike in Testudo.

The third layer of muscles on the ventral side of the neck, the *intertransversarii colli obliqui* present in Lissemys is absent in Testudo, owing again to the restricted movement of the neck.

The *trachelomastoideus* (Fig. 4) has a broad origin from the mid-ventral line of the body of the first and second cervical vertebræ and from the outer side of the inner muscle belly of the *longus colli* of the third cervical vertebra. All the fibres run obliquely outwards and get inserted on the inner surface of the squamosal and the posterior border of the pterygoid. But in Lissemys (George and Shah, 1954) this muscle arises from the lateral border of the second cervical and the diapophysis of the third cervical vertebræ and the fibres run obliquely outwards and converge into a narrow belly which gets inserted on the medial surface of the posterior end of the squamosal. This muscle in both the animals acts as the lateral rotator of the head.

Movements of the Head and Neck

The Chelonia among the reptiles possess a singularly long and powerful neck exhibiting a variety of movements. The principal movements of protraction and retraction of the neck together with those of the limbs play the part of the rib movements in other vertebrates of forcing the entry and exit of air into and from the lungs for respiration. In our prevoius paper (1954) on Lissemys we have given an account of the various movements of the head and neck and the muscles responsible for them. The same movements are present in Testudo also but in a considerably restricted and subdued manner.

When Lissemys is turned turtle, it rights itself quite elegantly with the help of its head and limbs (George and Shah, 1954). But Testudo is unable to do so. We kept several specimens in an upside-down position and watched for days together and found that they could not right themselves to their normal position. This unfortunate condition in Testudo is due to the neck having undergone an abrupt bend which has already been mentioned, as a result of the animal acquiring the massive

dome-shaped carapace that is unsuitable for swimming.   If such a carapace came up as a mutation, it may be considered as a salient factor in the evolution of completely terrestrial Chelonia (tortoises).

Summary

1.   An account of the muscles of the head and neck of the familiar Indian tortoise *Testudo elegans* is given.   In addition to the characteristic modifications in the myology pertaining to the head and neck of this animal that distinguish it from other reptilian groups, certain fundamental differences that strike a contrast from another chelonian, Lissemys, described by us earlier (1954), have been noted. These differences in Testudo and Lissemys could be attributed to the restricted movements of the head and neck in the former.

2.   The bony transverse bar, the jugulo-quadrate bar of the skull though present in Testudo does not divide the primitive *adductor mandibulæ externus* into an upper *temporalis* and lower *masseter*, as in Lissemys.

3.   The *intermandibularis, geniohyoideus, genioglossus, hypoglossus, hypoglosso-glossus* and the *hypoglosso-hyoideus* muscles which are chiefly responsible for the throat movements and the movements of the tongue, are present.   The *genioglossus* is a single muscle unlike the one in Lissemys which consists of two, the *genioglossus externus* and the *genioglossus internus*.

4.   The *constrictor colli* forms a more or less complete muscular envelope round the whole of the neck unlike in Lissemys where it is restricted only to the anterior half of the neck.

5.   The *spinalis cervico-capitis* consists of five parts instead of four in Lissemys. The presence of an additional part in Testudo is perhaps to support the neck which has undergone a severe bend at this position.

6.   The *semispinalis* and the *longissimus cervicis* which in Lissemys exist as a complicated system of muscles linked up with each other for a characteristically varied and powerful muscular action, occur in Testudo as simple muscles showing the primitive segmental arrangement.

7.   The *rectus cervicis* remains as a single muscle even anterior to the tendinous intersection unlike in Lissemys where the muscle is divided anteriorly into three parts.

8.   The *retrachens capitis collique*, in Testudo is a composite muscle formed by the *sterno-cleido-mastoid* derived from the primitive *rectus capitis cervico-plastralis* and the *longus colli* series of muscles.

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THE DIGESTIVE SYSTEM OF *PANULIRUS POLYPHAGUS* (HERBST)

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THE alimentary canal in the Crustacea is divided into three regions—the foregut, midgut and hindgut. These regions are developed from the three embryonic rudiments —the stomodaeum, the mesenteron and the proctodaeum respectively. The foregut consists of the buccal cavity, the œsophagus and the stomach. It is known that the extent of the midgut varies in the Decapoda. In Panulirus the midgut consists of a very small region behind the pyloric stomach and into it the hepatic ducts open. The hindgut consists of a long narrow tube extending from a ridge which demarcates the end of the midgut. The foregut and the hindgut are lined by a cuticle which is continuous with the cuticular covering of the integument. The short midgut is the only region which is devoid of cuticle and the alimentary canal very easily breaks at this region on handling. Except for the œsophagus which is placed somewhat vertically the rest of the alimentary canal is a long horizontal tube running the whole length of the body along the middle line.

The Foregut

The mouth is situated between the mandibles on the ventral side of the head region. In front the labrum overlaps the mouth as well as the incisor processes of the mandibles. The bilobed metastoma forms the posterior boundary of the mouth.

The mouth leads into a short wide tube—the œsophagus. It ascends upwards and dilates into a large sac—the stomach. The œsophagus presents a laterally compressed lumen, the lining of which is thrown into a few longitudinal folds. Anteriorly, that is on the dorsal side in transverse section, there are three folds, a median big fold with a smaller one on either side. The lateral walls form a large fold one on either side. Posteriorly, that is on the ventral side in transverse section,

the wall is thrown into a single narrow fold. . In the last portion of the œsophagus where it joins the stomach, the folds increase in number as the primary ones divide. The disposition of the folds in this part is such that they close the opening of the œsophagus into the stomach, so much so that the food has to be forced into the stomach.

The œsophageal wall shows the following layers (fig. 9, A). Externally there is an investing sheath which includes connective tissue and a thin layer of circular muscles, then a thick layer of longitudinal muscles and a layer of epithelial cells which secrete a cuticle. The outer investing sheath of the œsophagus is thick and is permeated with considerable amount of fatty tissue. The œsophagus leads into a spaceous bag—the stomach.

The stomach :—It occupies the whole length of the cephalic region and is divided into two parts, an anterior large chamber, the cardiac stomach, and a small posterior one, the pyloric stomach. When viewed dorsally or laterally the two divisions of the stomach can be made out by the presence of a slight depression between them. The cuticular lining of the cardiac stomach, in its hind portion forms a number of ossicles which constitute a remarkable apparatus, the gastric mill. In the pyloric region, the cuticle develops bristles which forms a filtering mechanism.

The cardiac chamber :—The anterior half of the cardiac stomach is a pouch in which the food gets accumulated. Regurgitation of food from cardiac stomach into the œsophagus is prevented by the muscular folds (already referred to) at the termination of the œsophagus.

The gastric mill :—The gastric mill is composed principally of eight ossicles, (Figs. 1, 2B). The largest of them is a triangular bar which runs transversely on the roof of the cardiac stomach. This is called the cardiac or meso-cardiac ossicle. The roof of the stomach in this region is sunk in a regular backwardly descending slope. It then ascends sharply and is again deflected backwards. As a result of this a backwardly sloping depression is formed. Placed behind at right angles to the cardiac ossicle is a median piece, the urocardiac ossicle. A Y-shaped ossicle is situated on the ascending back-wall of the above-mentioned depression. It is the pre-pyloric ossicle. Thus the posterior end of the urocardiac ossicle and the lower end of the Y of the pre-pyloric ossicle come to meet at the bottom of the depression. At this junction a well-formed tooth is developed on the urocardiac ossicle. This is called the median tooth. This tooth is slightly bifurcated and curved backwards projecting into the lumen of the stomach. The surface of the urocardiac ossicle facing the interior of the stomach is thrown into ridges. If the upper divided portion of the pre-pyloric is tilted backwards, the urocardiac ossicle along with the median tooth is moved somewhat forwards. Thus a forward and backward shifting of the median tooth is rendered possible.
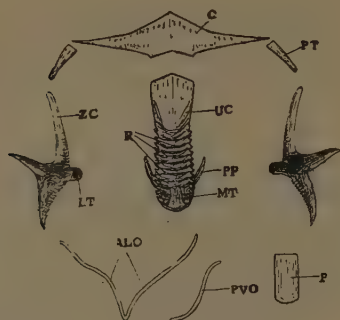
Fig. 1.   The various ossicles of the Gastric mill of Panulirus.
ALO, accessory lateral ossicle ; *C*, cardiac ossicle ; *LT*, lateral tooth ; *MT*, median tooth ; *P*, pyloric ossicle ; *PP*, pre-pyloric ossicle ; *PT*, ptero-cardiac ossicle ; *PVO*, posterior ventral ossicle ; *R*, ridges on the inner surface of the urocardiac ossicle ; *UC*, urocardiac ossicle ; *ZC*, zygocardiac ossicle.

Placed lateral to the cardiac ossicle is a pair of ptero-cardiac ossicles, one on either side. These ossicles are tiny and thin, rod-like and poorly chitinised. They are often overlooked on account of their small size. The ptero-cardiac ossicles are wedged in between the lateral extremeties of the cardiac ossicle and the anterior ends of a pair of zygocardiac ossicles which project backwards.

The zygocardiac ossicles are placed laterally on either side of the cardiac stomach. Each of them consists of two portions namely an anterior rod-like portion, and a posterior three-rayed one. The three rays are unequal in size. The inner ray is shorter than the other two and it bears a blunt conspicuous tooth. The posterior ray is the longest and stoutest and its ventral side is serrated showing not less than thirteen smaller, but well marked teeth. The upper ray is directed more or less vertically and it bears no teeth. The three-rayed structure is somewhat concave and the concavity faces the lumen of the stomach. The disposition and nature of the zygocardiacs resemble those of similar pieces in Asactus.

Behind the zygocardiacs is a pair of V-shaped slender bars known as the accessory lateral ossicles. The anteriorly directed limb of the ossicle is located behind the lateral margin of the cardiac stomach and its tip articulates with the three-rayed portion of the zygocardiac ossicle, while the posterior limb lies on the lateral wall of the pyloric chamber.

Proceeding from the junction of the anterior and posterior handles of the accessory lateral ossicles and running vertically downwards along the ventral wall of the stomach is a delicate rod. It is known as the posterior ventral ossicle. The posterior ventral ossicle along its hind end is articulated with the supra-ampullary fold of the pyloric stomach which will be described later.
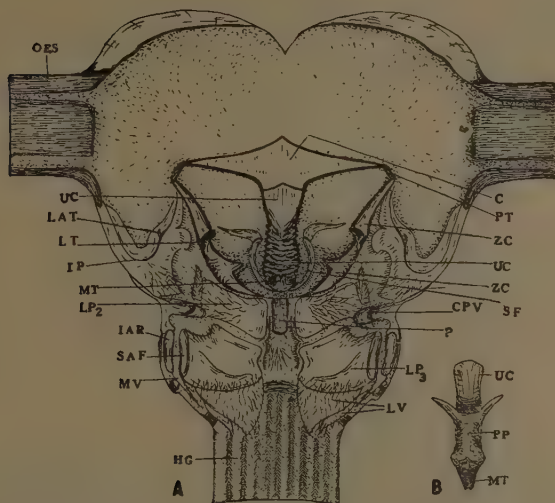
Fig. 2. A.—The stomach of Panulirus laid open along the Mid-ventral line.
B.—The urocardiac and pre-pyloric ossicles dissected out (dorsal view).

C, cardiac ossicle ; CPV, cardio-pyloric valve ; HG, hind-gut ; IAR, inter-ampullary ridge ; LAT, lateral accessory tooth ; LP, LP$_2$, LP$_3$, lateral pads ; LT, lateral tooth ; LV, lateral valves ; MT, median tooth ; MV, median valve ; OES, œsophagus ; P, pyloric ossicle ; PP, pre-pyloric ossicle ; PT, ptero-cardic ossicle ; SAF, supra-ampullary fold ; SF, semicircular fold ; UC urocardiac ossicle ; ZC, zygocardiac ossicle.

Behind the pre-pyloric ossicle, the roof of the stomach slopes backwards. On this slope is found a small rectangular piece very feebly calcified. It is the pyloric ossicle.

There are three pairs of pads developed on the ventro-lateral region of the cardiac stomach adjacent to the pyloric chamber. Of these the anterior-most consists of a pair of large triangular pads, the posterior margins of which are strengthened by the posterior ventral ossicles. Above these are two smaller pads lying against the lateral tooth. Below the serrated margin of the zygocardiac ossicles lie the other pair of oblong pads with a notch-like bit adjacent to the serrated margin. This pair lies adjacent to and in front of a pair of pads developed on the lateral walls of the pyloric chamber. The accessory lateral ossicles separate these pads from the pyloric ones. These pads are beset with setæ (Fig. 2A, 3).

When the stomach is slit open on the mid-dorsal line it is seen that the floor of the cardiac chamber is short and narrow and is pushed into its cavity so as to give rise to a projecting spoon-like process called the cardio-pyloric valve (Fig. 4). The handle of the spoon is anterior and is bifurcated at its anterior tip, while the posterior broad portion projects into the pyloric chamber. It bears a deep groove

along its length on its mid-dorsal side. On either side the handle of the valve is strengthened by the posterior ventral ossicles.   The groove is fringed with a row of long setæ.   The posterior thickened projecting portion of the spoon is also densely beset with setæ.
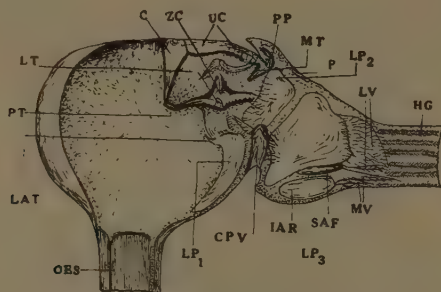

Fig. 3.   A lateral view of the internal structure of the stomach of Panulirus.
C, cardiac osscile ; *CPV*, cardio-pyloric valve ; *HG*, hind-gut ; *IAR*, inter-ampullary ridge ; *LAT*. lateral accessory tooth ; *LP* 1, *LP* 2, *LP* 3, lateral pads ; *LT*, lateral tooth ; *LV*, lateral valves ; *MT*, median tooth ; *MV*, median valve ; *OES*, œsophagus ; *P*, pyloric ossicle ; *PP*, pre-pyloric ossicle ; *PT*. ptero-cardiac osscile ; *SAF*, supra-ampullary fold ; *UC*, urocardiac ossicle ; *ZC*, zygocardiac ossicle.

The pyloric chamber :—The pyloric chamber is only about one-fourth of the size of the cardiac stomach.   Laterally it is bounded by two thick cuticular pads, already referred to. The lower margins of the pads are thickneed to form cushions known as the supra-ampullary folds.   The floor of the pyloric chamber is raised upwards between the supra-ampullary folds to form a chitinised ridge, the inter-ampullary ridge.   The two supra-ampullary folds along with the inter-ampullary ridge constitute what is known as the pyloric filter or strainer.   The efficiency of this apparatus can be recognised by the complicated structure it presents (Fig. 5A). The thick cuticular pads bear setæ on their anterior and posterior margins.   The convex surfaces of the supra-ampullary folds are densely beset with long bristles. The concave lateral surfaces of the inter-ampullary ridge bear a number of parallel longitudinal ridges.   Each of these ridges bears a row of comb-like bristles which project over the groove adjacent to it.   Besides the edges of the ridge are fringed with very fine setæ.   The supra-ampullary folds are situated so close to the inter-ampullary ridge, that practically only a small lumen is left between them.

The pyloric chamber is very narrow since it is laterally flattened.   A transverse section passing through the posterior region of the pyloric chamber shows a three-rayed narrow fissure.   Since the setiferous passage allows only finely ground food, the pyloric chamber works as a strainer.   Behind the filtering apparatus described above, there project from the lateral walls of the pyloric chamber two pairs of unequal broadly triangular flaps.   There is also a pair of unequal narrowly
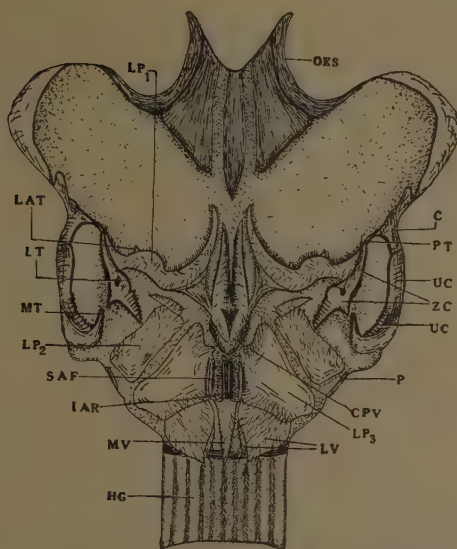
FIG. 4. The stomach of Panulirus laid open along the Mid-dorsal line.
    C, cardiac ossicle ; CPV, cardio-pyloric valve ; HG, hind-gut ; IAR, inter-ampullary ridge ;
LAT, lateral accessory tooth ; LP1, LP2, LP3, lateral pads ; LT, lateral tooth ; LV, lateral
valves ; MT, median tooth ; MV, median valve ; OES, œsophagus ; P, pyloric ossicle ; PT,
ptero-cardiac ossicle ; SAF, supra-ampullary fold ; UC, urocardiac ossicle ; ZC, zygocardiac
ossicle.
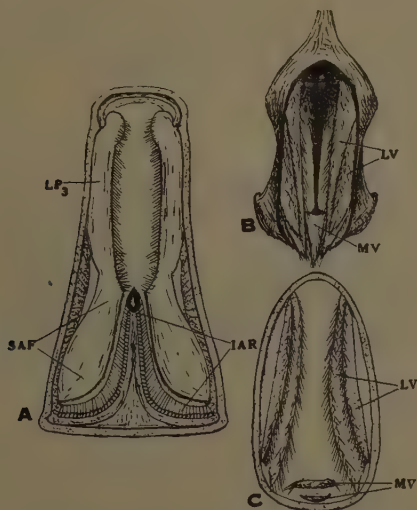
FIG. 5. The Structure of the Stomach of Panulirus.
    A.   T.S. through the Posterior Region of the Pyloric Stomach.
    B.   A view of the interior of the Pyloric Stomach from behind.
    C.   T.S. of the Midgut to show the Pylorico-intestinal Valve.
    IAR, inter-ampullary ridge ; LP 3, lateral pad ; LV, lateral valve ; MV, median valve ;
SAF, supra-ampullary fold.

triangular flaps projecting from the mid-ventral region of the pyloric chamber. The upper member of this pair, along with the two lateral pairs, projects beyond the pyloric chamber, while the lower one is smaller and does not reach the midgut. These flaps together constitute the pylorico-intestinal valve (Fig. 5B, C). As a consequence of the projecting flaps from the lateral walls of the pyloric chamber, the cavity of the midgut is divided into a median and two lateral chambers. The hepatic ducts open into the lateral chambers.

The inter-ampullary ridge at the junction of the pyloric chamber and midgut has a skeletal support of chitin which apparently even extends as a ring throughout the posterior rim of the pyloric chamber.

The gastric mill is worked by a complex system of extrinsic and intrinsic muscles (Fig. 6).   Their classification is based on that of Huxley for Astacus.

The Extrinsic Muscles of the Stomach

The extrinsic muscles consist of the following pairs (Fig. 7) :—

1. The anterior gastric muscles are a pair of long and strong muscle bands; they arise from the carapace between the post-orbital spines and terminate on the hind margin of the cradiac ossicle in the region in front of the urocardiac ossicle.

2. The posterior gastric muscles like the anterior gastric muscles are a pair of long bands; they connect the lateral extremities of the pre-pyloric ossicle as well as the dorsal wall of the posterior half of the cardiac stomach with the mid-portion of the carapace slightly in front of the cervical groove.
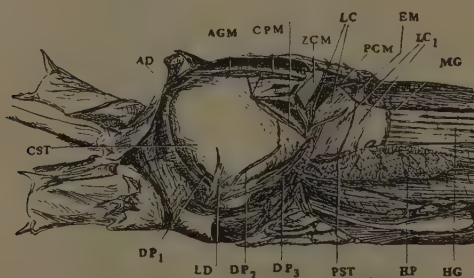
Fig. 6.   A dissection of Panulirus from the left side showing the Gastric Muscles.
    AD, anterior dilator muscle ; *AGM*, anterior gastric muscle ; *CST*, cardiac stomach ; *CPM*, cardio-pyloric muscle ; *DP* 1, anterior depressor muscle ; *DP* 2, median depressor muscle ; *DP* 3, posterior depressor muscle ; *EM*, elevator muscle ; *HG*, hindgut ; *HP*, hepatopancreas ; *LC*, the lesser constrictor muscles of the cardiac stomach ; *LC*1, the lesser constrictor muscles of the pyloric stomach ;   *LD*, lateral dilator muscle ; *MG*, midgut ; *PGM*, posterior gastric muscle ; *PST*, pyloric stomach ; *ZCM*, zygo-cardiac muscle.
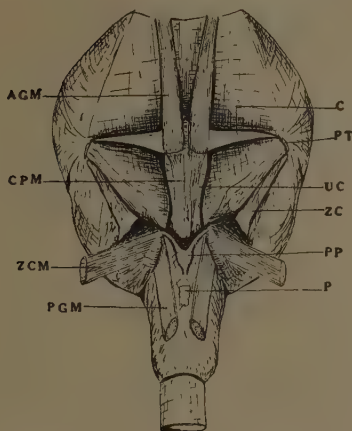
FIG. 7.   Dorsal view of the Extrinsic Muscles of the Cardiac Chamber of Panulirus.
AGM, anterior gastric muscle ; *C*, cardiac ossicle ; *CPM*, cardio-pyloric muscle ; *P*, pyloric ossicle ; *PP*, pre-pyloric ossicle ; *PGM*, posterior gastric muscle ; *PT*, ptero-cardiac ossicle ; *UC*, urocardiac ossicle ; *ZC*, zygocardiac ossicle ; *ZCM*, zygocardiac muscle.

3.   The zygocardiac muscles are a pair of short bands connecting the three-rayed portion of the zygocardiac ossicle to the lateral portion of the carapace in front of the cervical groove.

4.   The elevator muscles which are a pair of delicate muscle bands joining the roof of the pyloric chamber to the carapace at the place where the posterior gastric muscles join it.

5.   The depressor muscles are three pairs, which arise from the front edge of the extension of the epistoma over the antennary socket and are disposed as follows :—

   (a)   Anteriorly a pair of slender muscle bands which passing upwards from the origin takes a forward course and is inserted on the fronto-lateral wall of the cardiac sac.

   (b)   Behind another pair of slender strips joins the posterior wall of the cardiac sac and the sternal skeleton just near the first pair of depressor muscles.

   (c)   Posteriorly a pair of long strips runs between the junction of the ventral wall of the cardiac and that of the pyloric stomach, and the sternal skeleton near the median pair of depressor muscles.

6.   There are two pairs of dilator muscles as described below :—

   (a)   The dorsal anterior dilator muscles which form a small pair.

originate from the inner surface of the carapace just behind the orbit, and spread out on the anterior surface of the cardiac stomach ;

(b)   The lateral dilator muscles are well-developed.   Each of them originates from the front edge of the epistoma of the antennary socket above the origin of the three depressor muscles, and spreads out on the lateral wall of the cardiac stomach.

The Intrinsic Muscles of the Stomach

The intrinsic muscles consist of the cardiac and pyloric constrictors and the cardio-pyloric muscles (Fig. 8).

The cardiac constrictors :—The cardiac constrictors consist of the great constrictor and a pair of lesser constrictors.

1.   The great constrictor is a large sheet investing the postero-ventral wall of the cardiac sac and it connects the postero-ventral ossicles which run parallel with one another.

2.   The lesser constrictors comprise two pairs of delicate sheets of muscles which on contraction depress the upper wall of the cardiac stomach. Their disposition is described below.
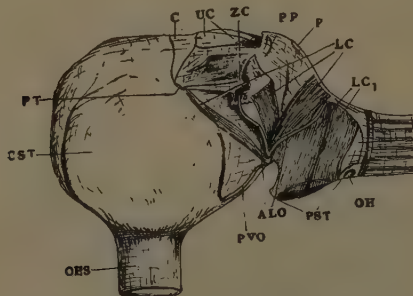
Fig. 8.   A dissection of Panulirus from the left side showing the Intrinsic Gastric Muscles. *ALO*, accessory lateral ossicle ; *C*, cardiac ossicle ; *CST*, cardiac stomach ; *LC*, the lesser constrictor muscles of the cardiac stomach ; *LC* 1, the lesser constrictor muscles of the pyloric stomach ; *OES*, œsophagus ; *OH*, opening of the hepatic duct ; *P*, pyloric ossicle ; *PP*, pre-pyloric ossicle ; *PT*, ptero-cardiac ossicle ; *PST*, pyloric stomach ; *PVO*, posterior ventral ossicle ; *UC*, urocardiac ossicle ; *ZC*, zygocardiac ossicle.

(a)   One of these pairs originates on the ventro-lateral wall of the cardiac stomach at the angle of the V-shaped accessory lateral ossicle.   Each of them after ascending upwards splits into two,

of which the anterior branch terminates on the zygocardiac ossicle and the posterior one on the pyloric ossicle.

(*b*) The second pair of the lesser constrictors is a pair of muscle strips each of which originates from the ventro-lateral wall of the cardiac stomach outside the angle of the accessory lateral ossicle and joins the anterior tip of the zygocardiac ossicle.

The pyloric constrictors:—They are also made up of the great constrictor and lesser constrictors.

1. The great constrictor is a small sheet of muscles investing the internal wall of the pyloric chamber. The fibres fill up the gap between the two walls of the pyloric chamber.

2. The lesser constrictors are a pair of delicate sheets of muscles investing the side walls of the pyloric chamber.

The cardio-pyloric muscles : —They are a pair of strands running between the hinder margin of the cardiac ossicle and in front of the junction of the urocardiac and pre-pyloric ossicles. Thus they lie in the depression, sloping backwards on the roof of the cardiac stomach.

Action of the stomach muscles :—The various muscles described above, work in unison to bring together the teeth, so as to employ them to grind the stomach contents and to deploy the various valves to strain the ground food material. Those that play the major part in the working of the gastric mill are the anterior and posterior gastric muscles, the intrinsic muscles, and the cardio-pyloric muscles.

When the anterior gastric muscles contract, they will pull forwards the median dorsal wall of the cardiac chamber. Consequently, the median tooth will share the same shifting of position. The contraction of the posterior gastric muscles will pull the top portion of the pre-pyloric backwards, but push its other end forwards and slightly downwards. This movement will help indirectly the movement of the median tooth forwards and downwards. When these two pairs of muscles contract and relax in unison, the median grinding tooth is made to move forwards and backwards along the arc of a circle. This movement will be further faciliated and made more harmonious by the contraction and relaxation of the cardio-pyloric muscles. This is the explanation given by Huxley for Astacus.

The contraction of the lesser constrictor muscles, one pair connecting the anterior ends of the zygocardiac ossicles and the other the triradiate portion of the zygocardiacs pushes the zygocardiacs inwards and downwards towards the mid-dorsal line. The result is that the lateral teeth borne upon the outer angle of the zygocardiac ossicles are brought to the level of the median tooth to clash against it. The sum total of the contraction and relaxation of the gastric muscles is to approximate the three teeth towards one another and draw them back. It will also make

the lateral teeth rub against the serration of the urocardiac ossicle. It should be mentioned here that when the teeth meet, they meet in front of the cardio-pyloric valve. The food which has found its way into the posterior part of the cardiac stomach is thus caught between these teeth and broken into fragments.

The functions of the other muscles which control the movement of the other parts of the stomach are described below.

The first two pairs of depressor muscles by their contraction lower the side walls of the stomach. Consequently the cavity of the stomach is decreased from top to bottom and slightly increased from side to side, so much so that food is brought to the centre of the stomach. The contraction of the last pair of depressors serve to lower the ventral wall of the pyloric stomach. The elevator pair of muscles serves to raise the roof of the pyloric stomach. These movements help to fill or empty the cavity of the pyloric stomach as the case may be.

The great constrictor of the cardiac stomach has a direct control over the cardio-pyloric valve and apparently it helps to push the food forwards and upwards. The constrictors of the lateral wall of the posterior half of the cardiac division serve to constrict the opening into the pyloric chamber and thus help to push the food back. The constrictors of the lateral wall of the pyloric stomach by their contraction serve to reduce the cavity of the pyloric stomach and thus push the food further back. The disposition of the anterior and lateral dilator muscles is such that, they help in keeping the cardiac stomach dilated from the anterior as well as the lateral sides.

The Midgut

As already stated the midgut is short and begins from the pylorico-intestinal valve mentioned above. Its posterior limit is marked externally by a circumferential ridge. The midgut is comparatively narrow and the anterior part of its cavity is partially occupied by the projecting flaps of the pylorico-intestinal valve, which prevent the regurgitation of the food back into the pyloric chamber from the intestine and also serve to direct large pieces of food coming through the pyloric stomach straight to the midgut.

The Hindgut

The hindgut is a long tube. Its lining is thrown into many longitudinal folds. A section through the anterior region of the hindgut shows the following parts (fig. 9B). Externally it is bounded by the investing tissue sheet. Next to it is a very thin layer of circular fibres and a very thick layer of longitudinal muscle fibres, a basement membrane, and an epithelial layer its cuticle bearing bristles. The mid-region of the hindgut possesses the same layers but the folds are less prominent (fig. 9C). The last portion of the hindgut narrows down considerably and the folds become very prominent again (fig. 9D). The layer of the circular

muscle fibres is very well developed here, since by their contraction the fæces is ejected out. The intestine opens to the outside through the anus which is a longitudinal slit situated ventrally at the base of the telson.
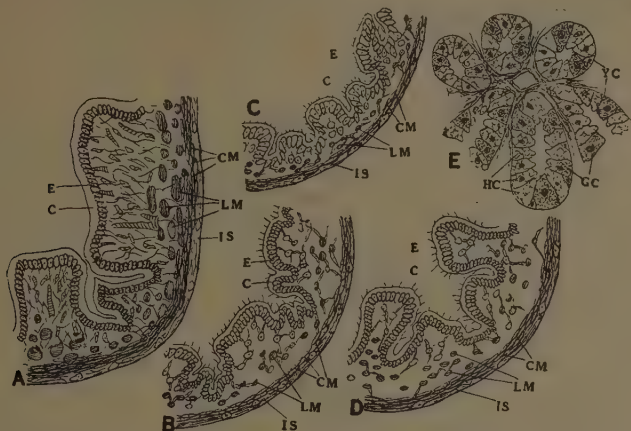
FIG. 9. The Histology of the Hindgut and Hepatopancreas of Panulirus.
A.   T.S. of the œsophagus.
B.   T.S. of the Anterior Region of the Hindgut.
C.   T.S. of the Mid Region of the Hindgut.
D.   T.S. of the Posterior Region of the Hindgut.
E.   T.S. of the Hepatopancreas.
C, cuticle ; CM, circular muscles; GC, granular cells; HC, hepatic cells ; IS, investing sheath ; LM, longitudinal muscles ; YC, young cells.

The Hepatopancreas

The hepatopancreas consists of a pair of massive highly branched glands lying one on either side of the alimentary canal.   In the body cavity they extend anteriorly as far as the mandibles and posteriorly to the level of the fourth pair of walking legs and hence occupy a considerable part of the cephalo-thoracic cavity.   In some they are orange-yellow in colour and in others a shade darker.   The lobules of the gland are arranged all around the lobular duct.   These ducts join together to form the main duct which as already mentioned opens into the midgut just behind the pyloric filter.   Externally the lobule is covered over by a very fine outer coat, the *tunica propria* formed of connective tissue with a delicate network of circular and longitudinal muscle fibres.   Next is a very thin layer of tall epithelial cells.

The epithelium consists of two types of cells which are described below (fig. 9E).

1.   The granular cells each having a nucleus and granular cytoplasm.   These are ferment cells which are very few and are found scattered among the other epithelial cells, they secret the digestive enzymes.

2.   The hepatic cells resemble the granular cells in shape but are larger. Moreover, these are vacuolated—their protoplasm being filled with fat globules.

In addition to these cells there are also the basal or replacing cells which are young cells of various shapes.

The Food and Its Digestion

When the stomach is laid open, at times it is found to be filled with pieces of fish bone, molluscan shells and occasionally with some smaller forms of crustacea. Vegetable matter and sand grains are also met with in the stomach.   A yellow fluid is often mixed with the stomach contents.   As pointed out by Huxley for Astacus, this fluid is obviously the hepatopancreatic secretion which finds its way through the pyloric filter into the cardiac stomach, mixes with the food and renders it soft and pliable for action by the gastric mill.

The food that is tirturated by the gastric mill has to pass the valves at the end of the cardiac chamber on its way to the pyloric chamber.   If not in the form of fine powder, the food is caught in the pockets of the posterior part of the cardiac chamber and is sent back by the movements of the bristles to the anterior part of the cardiac stomach.   The median cardio-pyloric valve which projects into the pyloric chamber also obstructs the free passage of food particles into the latter.   The coarser particles along with some fluid can overflow the valve and find their way to the pyloric chamber.   After passing beyond this valve the food has to pass the pyloric strainer.   The latter on account of the closer arrangement of its walls and the projecting bristles forms an efficient filter and food can pass through this only in very minutely divided state.

In addition to the powdered food flat pieces of bone, and molluscan shells have been occasionally met with in the hindgut ; it means they can pass through the upper ray of the three-rayed fissure of the pyloric chamber as well as between the pylorico-intestinal flaps.

Yonge who has studied the physiology of digestion and absorption in the Norway lobster, *Nephrops norwegicus* finds that the hepatopancreas secretes digestive enzymes which are capable of digesting carbohydrates, proteins and fats as noted in Astacus by Huxley.   He also finds that the secretion is poured into the midgut and is conveyed both into the pyloric and cardiac chambers of the stomach.   There it mixes with the food and dissolves it.   The dissolved food material is strained through the bristles of the combed ridges and is passed into the lobules of the hepatopancreas.   The dissolved materials are thus absorbed by the cells of the hepatopancreas.   In Panulirus, the water extract of the hepatopancreas is found to be slightly acidic.   The acidity of the secretion is limited to the entrance of the lobules where the food material is somewhat softened.   Tryptic digestion seems mainly

confined to the lobules where the juice is alkaline. Carbohydrates are acted on even from the stomach. The hepatopancreas serves also as an important storage organ. There is an erroneous impression that this organ stores up reserves in the form of glycogen. The amount of glycogen in the hepatopancreas was found to be negligible. T. Huxley, however, has mentioned that no more glycogen is found in the hepatopancreas of Astacus than is met with in other tissues.

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THE HOMOLOGY OF ARTERIAL ARCHES IN THE AMNIOTES

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THE homology of the aortic arches in the tetrapods has been a theme of great interest to zoologists ever since the study of comparative anatomy became recognised as a branch of human knowledge. The modifications of the aortic arches of the fishes became necessary due to the changes from branchial to pulmonary respiration and those of the heart due to the separation of the venous and arterial blood flow in that organ. These changes which started in the Amphibia reached their culmination in the birds and mammals.

The conus arteriosus, a structure so characteristic of the elasmobranchs, Dipnoi and amphibians is lost in the other groups of vertebrates, though some suppose that it is replaced by the bulbus arteriosus, a non-contractile part of the aorta, for example in Amia and other Osteichthys (Gegenbaur, 1891). What has been suspected as a remnant of the conus arteriosus with a couple of transverse rows of valves has been reported from Albula (Boas, 1880) and in Tarpon and Megalops (Senior, 1907). The disappeared conus has been supposed to have merged with the ventricle in the teleosts (Hoyer, 1900, Senior, 1907, and Smith, 1918). This view finds expression in text-books in the remark that " the conus arteriosus with its several rows of valves has become completely absorbed into the ventricle " (Shipley and Macbride, 1904).

On the other hand, while dealing with the arterial arches of reptiles it has become customary to explain the absence of the conus arteriosus by assuming that it " is completely divided by two spiral septa so that there are one pulmonary and two aortic channels opening from the ventricle " (Young, 1952).

The structure of the ventral aorta in frogs sheds some light on this subject. In the frog, *Rana tigrina* for example, the ventral aorta is divided first into two portions by a horizontal septum, the ventral of which becomes the systemico-carotid chamber and the dorsal which leads into the pulomonary arch, the pulmonary chamber. The ventral systemcio-carotid chamber a little beyond, becomes divided into a right and left part by a septum ; the former leads into the right systemico-carotid and the latter into the left systemic. Farther on, the cavity of the right systemico-carotid chamber becomes divided into that of the right systemic and those of the carotids. That such is the structure of the ventral aorta in *R. tigrina* was shown by Rao and Ramanna (1925) and confirmed by George and Varkey (1944). In the frog therefore all the

essentials of the condition of the arterial arches in reptiles, namely the existence of the three separate aortic channels of which the right systemico-carotid gives off the carotid arteries, is foreshadowed.

From the standpoint of comparative anatomy we are therefore tempted to assume that in the reptiles the conus arteriosus has been absorbed into the ventricle as is assumed in the case of the Actinopterygii, and the ventral aorta is resolved into three component arteries. Support for this view comes from the work of O'Donoghue (1920) on Sphenodon, in which the arterial trunks arise from a projection of the ventricle. This projection he regards as a remnant of the conus arteriosus. In the spiny-tailed lizard, Uromastix, however, Bhatia (1929) finds the right systemic opening first into the ventricle, while the left systemic and the pulmonary joining later. If the conus arteriosus is supposed to have been absorbed into the ventricle in reptiles and the aortic arches derived from the ventral aorta, it becomes unnecessary to assume the formation of two spiral septa in the conus of the ancestor to split it into three arteries. There is no evidence of two septa in the conus reported from any amphibian.

In the case of mammals and birds it is generally presumed that the left systemic arch is preserved in the former, and the right one in the birds. Since no material evidence is available from embryology to support the above presumption, and in the light of the interpretation given above, is it not better to regard that in both these groups it is the right systemico-carotid that is preserved and that the left systemic being superfluous, is lost ? If this is correct, the right systemico-carotid arch arising from the left ventricle goes to the right side in birds and to the left side in mammals. This variance may be attributed to a greater gradient to the left in mammals, and to the right in birds. The possible causes of such a diversity, however, remains obscure. It may also be mentioned in this connection that in medical literature, records of the occurrence in man of double aortic arches or of the aortic arch being on the right side as in birds, are not wanting (Walter and Sayles, 1949).

Summary

On comparative anatomical grounds it appears more reasonable to uphold the following theoretical assumptions on the homology of the arterial arches in the vertebrates :—

1. That in those groups in which the conus arteriosus is absent it has been absorbed by the ventricle.

2. That the aortic arches of reptiles, namely the pulmonary and the right and left systemic arches, have been derived from the resolution of the ventral aorta and not of the conus arteriosus.

3. That the aortic arches of mammals and birds are homologus, having originated from the right systemico-carotid arch of the reptiles.

Acknowledgment

We wish to express our thanks to Professor J. Z. Young who in a personal communication to the first author encouraged the persuit of this problem.

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HISTOLOGICAL FEATURES OF THE BREAST AND LEG MUSCLES OF BIRD AND BAT AND THEIR PHYSIOLOGICAL AND EVOLUTIONARY SIGNIFICANCE

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BULLARD (1912) has shown that the striated muscle contains interstitial granules and fat droplets. The granules were regarded by him to be albumino-lipoid in nature and the fat globules of neutral fat. Earlier, Bell (1911) had pointed out that the dark fibres of the muscle contained strongly refractive liposomes (fat droplets). Krause (1864) and Knoll (1891) had also drawn attention to the greater abundance of interstitial granules in red muscle than in pale one.

The senior author in two contributions (1947 and 1952) showed the presence of an abnormal amount of fat in the breast muscle of the pigeon and in another (1953) we pointed out the possibility of fat being utilized as fuel in long and sustained contraction of the flight muscle. It was also shown that a reduction of fat in the liver occurred as the contraction of the muscle was continued. The present study was undertaken to see if histological proof of the utilization of fat in muscle contraction could be found.

Materials and Methods

The material for this investigation consisted of the breast and leg muscles of the pigeon (*Columba livia*), a local kite (*Milvus migrans*), domestic fowl (*Gallus domesticus*) and a common microchiropterous local bat (*Rousettus lechinaulti*). The animal in each case was pithed and small pieces of the breast muscle (*pectoralis major* inbirds) of one side were cut out. Some of the pieces were placed in normal saline and others in Jackson's acetic carbol-sudan III stain. Pieces from a leg muscle were also cut out and put in both these solutions separately. The pectoral muscle of the other side in each case was electrically stimulated by a series of make and break shocks (about 50 per minute) with the current passed through the Du Bois induction coil from a battery cell of 4 volts, and adjusting the current at a minimum for a visible contraction and gradually increasing its intensity until the muscle was thoroughly fatigued. Then pieces were cut out from it and separately put in the fluids mentioned above.

To study the effect of fatigue on the structure of the muscle fibre, a pigeon was forced to fly repeatedly in a large hall till it was more or less fatigued. It was immediately pithed and a small piece of the breast muscle of one side was removed to study the muscle fibres. The breast muscle on the other side was then electrically

stimulated to total fatigue. A piece of this muscle was also examined. Some
pigeons were starved for a week and pieces of their breast muscles were also treated
in the manner mentioned above.

All the treated pieces of muscle were separately teased out and the fibres
were mounted. Microphotographs of the fibres were also made.

Results

On examining the stained as well as unstained preparations of muscle fibres,
the following histological features were noted :—

1. The breast muscle of the pigeon is red and is made up of two types of fibres
    One type consists of narrow fibres, the sarcoplasm of which is interspersed
    with translucent fat globules as well as opaque interstitial granules.   The
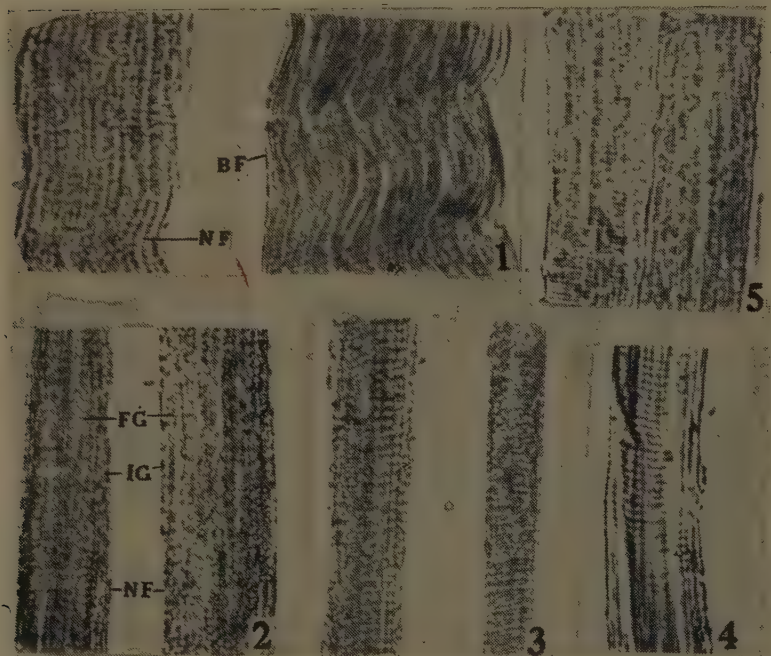
FIG. I.   Microphotographs showing the structure of the breast and leg muscle fibres ;
1.   Narrow and broad fibres of the pigeon breast muscle (x 600).
2.   Narrow fibres of the pigeon breast muscle before stimulation (x 600).
3.   Narrow fibres of the pigeon breast muscle after forced flight (x 600).
4.   Narrow fibre of the pigeon breast muscle after forced flight and stimulation
     (x 600).
5.   Narrow fibres of the pigeon breast muscle after starvation (x 600).
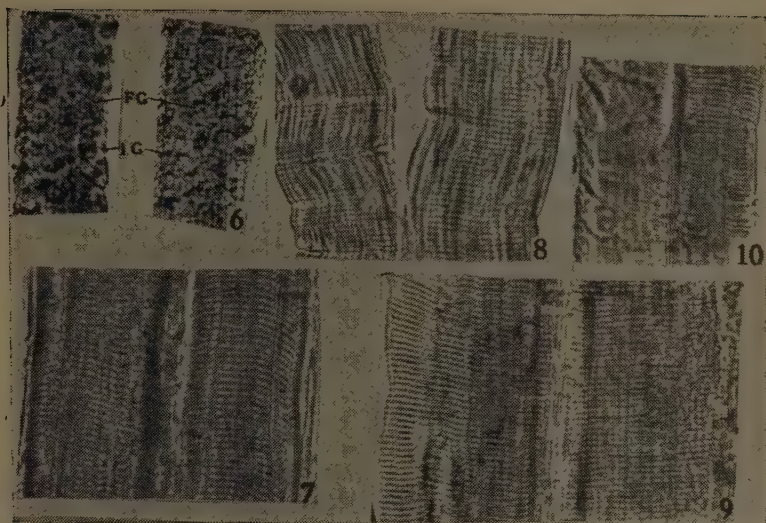
FIG. I—*Continued.*

6. Breast muscle fibres of the bat (x 475).
7. Leg muscle fibres of the fowl (x 475).
8. Leg muscle fibres of the pigeon (x 475).
9. Leg muscle fibres of the fowl (x 475).
10. Leg muscle fibres of the bat (x 475).

BF, broad fiber;
FG, fat globule;
IG, interstitial granule;
NF, narrow fiber.

granules appear to be of lipoprotein nature. In this fibre the striations characteristic of the skeletal muscle are obscured by these inclusions. The other type of fibre is broader, clearer in microscopic vision and the striations are well-marked on it. In this fibre the granules and globules characteristic of the first type are markedly fewer and the latter are minute. In the construction of the muscle a broad fibre is surrounded by a number of smaller ones, so much so that the latter are much more numerous than the former (Fig. I, 1).

2. The breast muscle of the kite is also red and is made up of the two types of fibres met with in the pigeon, but unlike in the latter the narrow type of fibre appears to be fewer in number than in the pigeon.

3. The breast muscle of the fowl is pale unlike the red-tinted ones of the pigeon and kite. The fibres are all of the broad type (Fig. I, 7).

4. The breast muscle of the bat is red and is made up solely of the narrow type of fibres, the broad type being absent. Moreover, the inclusions are numerically more than in the breast muscle of the pigeon, and present therefore a prominent crowded appearance (Fig. I, 6).

5. The leg muscles of all the above-mentioned animals consist of only the broad type of fibres. The fibres of the leg muscle of the fowl, however, are slightly reddish and possesses comparatively prominent striations (Fig. I, 8, 9, 10).

6. The narrow fat-loaded fibres of the breast muscle of the pigeon on forced, exhausting flight or electric stimulation gradually lose their fat globules and consequently their striations appear rather very prominent after prolonged stimulation (Fig. I, 3, 4).

7. The narrow fat-loaded breast muscle fibres from starved pigeons are also found to be depleted of their fat inclusions (Fig. I, 5).

Discussion

It was Bullard who gave us for the first time a clear picture of the muscle fibre inclusions. The present study has shown that two types of muscle fibres occur in the higher vertebrates, those richly laden with large fat globules in their sarcoplasm and others with only a sparse distribution of tiny globules. The presence of the fat-loaded fibres in the breast muscle of the pigeon in large numbers and the disappearance of the fat globules on stimulation and also in flight, give demonstrable support to our earlier finding (1953) on the utilization of fat in sustained muscular activity. This view gets support from the work of Weis-Fogh (1952) who maintained that fat constitutes the principal source of energy in the locust muscle and suggested that they make use of it in their migration. Bullard who noted the disappearance of fat globules in starvation came to the conclusion that fat in muscle fibres served as reserve food material. Our finding is that fat globules in the muscle fibre are first and foremost material for prolonged flight and their utilization during starvation is a secondary purpose. Otherwise, there is no reason why the domestic fowl should not have a store of fat in its flight or leg muscle.

It appears that the broad, less-fatty fibres around which the fat-loaded ones are grouped in the breast muscle of the pigeon, are the first to contract in flight, and as the muscle gets warmed up, the others also contract and thus gradually the fat in the fibres is utilized. For short flights therefore the glycogen load of the muscle might be readily used and that must also be the case in walking and running by the domestic fowl.

The soaring bird, kite, has also a high percentage of fat in its breast muscle, but the fat-loaded narrow fibres are less in number than in the breast muscle of the pigeon. The soaring flight is less taxing and goes on for long and the less number of fat-loaded fibres might meet the needs of soaring flight in the kite. Moreover, the sharp movements involved in the soaring flight are better met by the glycogen-loaded fibres which are also present in sufficient numbers in the kite breast muscle

The microchiropterous bats are for most of the time on their wings during their foraging at nights.   During such flights they have to expend a large amount of energy and that is supplied by the fat-loaded breast muscle fibres.

There is no need of fat-loaded fibres in the leg muscle. Even the domestic fowl which is a runner can never stand persistent persuit, which means that its leg muscle gets easily fatigued.   In the case of the wing muscles of the domestic fowl, the fat-loaded fibres are absent and that explains the limited flight propensities of the domestic fowl.

The over-all outcome of the present investigation is the light it throws on the evolution of the muscle fibre in vertebrates.   The ancestral and embryonic muscle fibre is the glycogen-deposited white one.   The amphibian and reptilian muscles are generally composed of white fibres only.   But in some fishes in addition to the white muscle, streaks of red muscle are also met with.   Alexander (1955) showed that the red muscle of fishes is metabolically superior to the white one, as it contains more proteins and fat and less water. Moreover, the myoglobin present in it supplies it with ready oxygen to facilitate oxidation.   The red muscle is met with extensively in birds and mammals. The emergence of the red fibre is the first stride in the physiological evolution of the muscle fibre.   The next evolutionary stride is the fat-loaded narrow red fibre.   This fibre is present in the breast muscle in good numbers in the kite, more in the pigeon and exclusively in the bat.

As the breast muscle of the pigeon with its majority of fat-loaded fibres represents an extreme of the red muscle evolution in birds, the breast muscle of the domestic fowl represents an extreme of the glycogen-loaded white fibre muscle. We have found (data so far unpublished) that the amount of glycogen in the breast muscle of the fowl is nearly double that of the breast muscle of the pigeon.

Summary

1.   Histologically the breast muscle of the pigeon is made up of two types of fibres, a narrow one studded with large globules of fat, and granules of lipoprotein nature, and a broad one with the inclusions very sparsely distributed and the fat globules minute.   The former type is much more numerous than the latter.

2.   The breast muscle of the local kite is also made up of the two types of fibres, but the narrow fat-loaded ones are fewer in number than the broad less-fatty ones.

3.   The narrow fat-loaded type of fibre is totally lacking in the breast muscle of the domestic fowl.

4.   The breast muscle of the common local microchiropterous bat is exclusively made up of the narrow type of fibre.   The inclusions of fat are, however, much more prominent in this animal.

5.   The leg muscle fibres in all these animals are of the broad less-fatty type. The leg muscle of the fowl is reddish and the striations are prominent.

6.   The fat-loaded fibres of the breast muscle lose their fat globules gradually on stimulation apparently due to their utilization in contraction.   The breast muscle of the starved pigeons is also found to be depleted of their fat globules. Most probably they have been used up to maintain life during starvation.   It is therefore concluded that fat inclusions are primarily useful during flight and serve secondarily as reserve store to maintain life during starvation.

7.   In the evolution of the muscle fibre, the emergence of the myoglobin-permeated more metabolic red type seems to be the first step.   The next step is the appearance of the fat-loaded narrow type as a variation from the broad less-fatty red fibre.

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THE LIPID CONTENT AND ITS REDUCTION IN THE MUSCLE AND LIVER DURING LONG AND SUSTAINED MUSCULAR ACTIVITY

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I T is an accepted fact now that the fuel for muscular activity is not always carbo-hydrate. That a considerable amount of protein is utilised during strenuous muscular exercise in man was shown by Cathcart and Brunett (1926) and that fat too may be utilized, by Krogh and Lindhard (1920) and Gemill (1940 and 1942). There seems to be some indirect evidence to show that fat is the chief fuel for energy during long and sustained muscular activity. Niemierko (1929) using spring frogs showed that prolonged stimulation of the muscle on one leg caused a decrease in its fat content up to 31 per cent. Buchwald and Cori (1931) using summer frogs found that the fatigued muscle of one leg contained 19·7 per cent less fatty acids than the resting muscle of the other side. Bloor and Snider (1934) showed that the phospholipid content of the muscles of a wild rabbit was higher than that of a laboratory one. Later, Bloor (1937) established that the phospholipid content of muscle increased as a result of muscular exercise and he (1940) also claimed that there exists an inheritance effect of exercise in the phospholipid and cholesterol content of muscle. Cruickshank and Kosterlitz (1941) have shown that rat heart can utilize fatty acids. Recently Weis-Fogh (1952) in his excellent paper observes that flying locusts utilize fat for muscular activity and as for the humming bird he has put a question mark. Fontaine and Hatey (1953) believed that fat is the chief fuel in migrating fishes (Salmon).

The senior author (1947 and 1952) has shown the presence of a large amount of fat in the flight muscle of the pigeon (Columba livia). Nair (1952) showed that the total solids in the *pectoralis major* muscle of the bird was higher than that in the *pectoralis minor*, thereby suggesting that the former is metabolically a more evolved muscle than the latter. The fat content of the *pectoralis major* has also been found to be greater and so also the iron content. He also observed that good fliers show a remarkably higher percentage of these substances in the muscle. Menon (1954) has shown that the glucose and fat in the blood of birds are the highest in the verte-brate series. George and Menon (1954) have suggested a physiological lag in the domestic fowl as the fat content in the blood is low and to which facts they ascribed its inability to fly.

Having been convinced on circumstantial evidence of the possibility of the utilization of fat by birds during flight, we tried to ascertain if a reduction of fat occurs either in the flight muscle or in the liver or in both in the pigeon when the flight muscles are stimulated. The results published in 1953 showed that the fat content in the breast muscle as well as liver gets reduced when the flight muscle is subjected to prolonged electrical stimulation.

In the present studies the experiments were extended to other birds and also to a microchiropterous bat. We also considered it desirable to ascertain in each case the amounts of free and bound lipids in the breast muscle and if a reduction of either or both of them occurs as a result of prolonged stimulation. For comparative purposes the treatment was also extended to the leg muscles.

Materials and Methods

The animals experimented with were the local parakeet (*Psittacula krameri*), kite. (*Milvus migrans*). pigeon. (*Columba livia*) domestic fowl (*Gallus domesticus*) and a bat (*Rousettus lechinaulti*). The animal was pithed and a small piece of the breast muscle (*Pectoralis major* in the case of the birds) on one side and a similar piece of the liver—both to be used as control. were cut out for the estimation of their lipid content. while the muscle on the other side was electrically stimulated by a series of make and break shocks (about 50 per minute) with the current passed through the Du Bois induction coil from a battery cell of $4 \cdot 0$ volts. and adjusting the current at a minimum for a visible contraction and then gradually increasing its intensity until the muscle was thoroughly fatigued. Then a small piece was cut out from the fatigued muscle, as well as from the liver.

Similarly a piece of the calf muscle of one leg was removed after the animal was pithed and then the same muscle of the other leg was stimulated as was done in the case of the breast muscle. A small piece of the fatigued muscle was also cut out.

The lipid content of the controls as well as of the stimulated muscle and of the liver before and after stimulation of the muscle was separately estimated.

It is found that after extraction with ether when the material is further treated with a mixture of ethanol and ether. a considerable amount of lipid is still obtained. It is assumed that ethereal extracts normally consist mostly of free lipid as neutral fat and free fatty acids, and a combustion of these should be mainly responsible for the reduction in the amount of lipid due to exercise of the muscle. We have ventured to call the first ethereal extract therefore as free lipid and the ether-soluble part of the ethanol-ether extract as total lipid. So. by total lipid we mean the total ether-soluble lipid which will consist of mostly of combustible fat.

For obtaining the free lipid we have used water-free ethyl ether. Since it was found that the amount of lipid obtained after dehydration by heat was slightly greater than when dehydrated under vacuum, it was suspected that heat does release some bound lipid. The material was therefore dehydrated first under vacuum. For obtaining the total lipid the material was first completely dried by heating first to 100°C and then gradually to 110°C till a constant weight was obtained. Then a 1 : 1 mixture of water-free ethyl ether and ethanol was used to extract the lipid. In either case the material was transferred to a fat-extraction thimble which was in turn placed in the extractor part of the soxhlet apparatus.

The percentage of lipid was calculated on the weight of the fresh material, but for muscle after fatigue the percentage of lipid was calculated first on its dry weight and then converted for fresh weight, because there is considerable loss of water when muscle is subjected to continuous contraction. The same procedure was adopted in the case of liver after the muscle was fatigued.

Results

The normal lipid content of the muscle and liver was found to vary within limits depending on the state of the animal. Such were also fat reduction values, since some specimens got fatigued sooner than others. It was therefore thought advisable to show the range in variation instead of mean values. The results are given in the following tables.

Discussion

Nineteen years ago Heilbrunn (1936) wrote " Although it is now generally realised that protoplasm is more than a solution of proteins, and that non-aqueous lipids are essential to its life, biologists and physiologists have scarcely given thought to the problem of how the lipids are related to the rest of the protoplasm. Most students of the cell are scarcely aware of the existence of the problem. And yet the pathologists have known for many years that the fats and fat-like substances of protoplasm are so bound or united to the proteins as to be for the most part non-recognizable in the living or stained cell. It is only when degeneration occurs that the lipids are freed from their union with protein. In such dengerated cells, fats previously concealed may occupy a large part of the cell volume. Obviously, such a freeing of lipids results in an impairment or loss of protoplasmic activity, and this bears witness to the importance of the lipids for the vital machinery." Even today our knowledge of the relationship of lipids to proteins remains obscure. In our present work, however, no attempts have been made to make this picture clearer. But we have found that for the muscle tissue three values of lipid are obtainable. The ether extractable lipid portion consisting of neutral fats and free fatty acids constitutes one value and this has been assumed to be the free lipid portion. The lipid portion extractable with a mixture of ether and ethanol has been taken as

TABLE I

The lipid content and its reduction in the pectoralis muscle*

Animal	Percentage of free lipid before stimulation	Percentage of free lipid after stimulation	Actual reduction of free lipid in gm. per 100 gm. of muscle	Percentage of reduction of free lipid	Percentage of total lipid before stimulation	Percentage of total lipid after stimulation	Actual reduction of total lipid in gm. per 100 gm. of muscle	Percentage of reduction of total lipid	Time taken for fatigue in minutes
Pigeon	2.50-3.00	2.00-2.10	0.50-0.90	20.00-30.00	4.21-4.73	3.72-3.80	0.49-0.93	11.63-19.66	15-20
Parakeet ...	3.30-9.80	2.46-2.48	0.82-1.34	24.85-35.26	4.90-5.76	4.05-4.56	0.85-1.20	17.35-20.84	18-22
Kite	2.83-3.20	2.56-2.85	0.27-0.35	9.50-10.94	4.50-5.00	4.25-4.61	0.25-0.39	5.50-7.80	8-10
Fowl	0.50-0.60	0.45-0.50	0.05-0.10	10.00-16.66	0.90-1.10	0.77-0.88	0.18-0.22	14.44 20.00	10-15
Bat	9.00-10.00	7.81-8.60	1.19-1.40	13.22-14.00	11.38-13.08	10.21-12.56	1.17-1.42	10.16-10.28	18-25

* In the birds the *pectoralis major* was used.

TABLE II

The lipid content and its reduction in the liver

Animal	Percentage of free lipid before stimulation	Percentage of free lipid after stimulation	Actual reduction of free lipid in gm. per 100 gm. of liver	Percentage of reduction of free lipid	Percentage of total lipid before stimulation	Percentage of total lipid after stimulation	Actual reduction of total lipid in gm. per 100 gm. of liver	Percentage of reduction of total lipid
Pigeon	2.50-3.50	2.25-3.00	0.25-0.50	10.00-14.29	3.81-4.50	3.05-3.97	0.26-0.53	7.80-11.78
Parakeet ...	4.20-4.70	3.40-3.80	0.71-0.90	16.90-19.14	5.20-5.90	4.50-5.02	0.70-0.88	13.46-14.91
Kite	3.20-3.90	2.80-3.42	0.40-0.48	12.30-12.50	4.10-4.90	3.71-4.40	0.30-0.50	9.51-1021
Fowl	2.90-3.20	2.50-2.81	0.31-0.39	10.69-12.19	2.98-3.40	2.68-3.00	0.30-0.40	10.07-11.77
Bat	5.60-6.00	5.20	0.40-0.80	7.14-13.33	0.53-7.24	6.11 6.41	0.42-0.83	0.48-11.47

TABLE III

The lipid content and its reduction in the leg muscle

Animal	Percentage of free lipid before stimulation	Percentage of free lipid after stimulation	Actual reduction of free lipid in gm. per 100 gm. of muscle	Percentage of reduction of free lipid	Percentage of total lipid before stimulation	Percentage of total lipid after stimulation	Actual reduction of total lipid in gm. per 100 gm. of muscle	Percentage of reduction of total lipid	Time taken for fatigue in minutes
Pigeon	1.70–2.00	1.44–1.70	0.26–0.30	15.00–15.30	1.94–2.25	1.69–1.95	0.25–0.30	12.88–13.33	8–10
Parakeet ...	2.00–2.60	1.60–2.03	0.40–0.57	20.00–21.11	2.30–2.90	1.94–2.34	0.36–0.56	15.65–19.32	8–12
Kite	1.40–1.57	1.21–1.35	0.19–0.22	13.57–14.02	1.90–1.97	1.69–1.74	0.21–0.23	11.05–11.68	5–8
Fowl	1.10–1.30	0.96–1.12	0.14 0.18	12.73–13.85	1.81–2.43	1.38–1.90	0.43–0.53	21.81–23.75	15–20
Bat	4.60–5.30	4.20–4.60	0.40–0.70	8.70–13.21	5.64–6.90	5.23–6.23	0.41–0.67	7.27–9.71	12–15

TABLE IV

Comparison of free and bound lipids per 100 gm. of pectoralis muscle, liver and leg muscle

		Pigeon	Parakeet	Kite	Fowl	Bat
Pectoralis muscle	Free lipid	2.50–3.00	3.30–3.80	2.80–3.20	0.50–0.60	9.00–10.00
	Bound lipid	1.71–1.73	1.60–1.96	1.63–1.80	0.40–0.50	2.38–3.98
Liver	Free lipid	2.50–3.50	4.20–4.70	3.20–3.90	2.90–3.20	5.60–6.00
	Bound lipid	0.81–1.00	1.00–1.20	0.90–1.00	0.08–0.20	0.98–1.24
Leg muscle	Free lipid	1.70–2.00	2.00–2.60	1.40–1.57	1.10–1.80	4.60–5.30
	Bound lipid	0.24–0.25	0.30	0.40–0.50	0.71–1.13	1.04–1.60

the total lipid. The difference between the two values is regarded as that of the bound lipid. What we have tried to understand is whether the lipid affected during continued muscular contraction in the muscle itself and also in the liver, is free lipid, bound lipid, or total lipid.

From the results obtained it is seen that the *pectoralis major* muscle of the domestic fowl contains the least amount of lipid both as free as well as bound, while the *pectoralis* of the bat the highest, and those of the parakeet, kite and pigeon come near to that of the bat in that order. The free and bound lipid figures are compared in table 4. The bound and free lipid values are more or less equal in the fowl, while in the other animals the free lipid values are definitely higher.

In the leg muscle the free lipid values are very much higher than those for the bound lipid in all the animals except the fowl, in which the corresponding value is only moderately high.

In the liver the free lipid values are very much higher than those for the bound lipid in all the animals.

In our previous paper (1953) it was shown that there is a reduction in the fat content of the *pectoralis major* of the pigeon as a result of continuous muscular contraction, and it has been suggested that fat gets transported to the muscle from the liver as it gets reduced in the former. The present investigation confirms the previous one and such a reduction has been noted in the case of the leg muscle also as a result of continuous stimulation.

It must be mentioned that the time taken for fatigue of the pectoralis muscle in the pigeon, parakeet and bat is from 15 to 25 minutes, whereas the kite muscle got fatigued in 8 to 10 minutes. Correspondingly the reduction of lipid was also less in the kite. This peculiarity may be due to the fact that the kite alone amongst these is adapted for soaring and the mechanism of energy utilization in soaring is different from that of flapping flight.

In the domestic fowl which is a poor flier the actual reduction of free and total lipid is little, but the total lipid reduction is greater, which is not so in the other animals. This suggests that in the case of the fowl, the bound lipid is utilized also on stimulation. This is the case both in the breast and leg muscle. This release of bound lipid is in some way connected with the acidity of the muscle. J. F. Manery *et al* (1935) have shown that the pectoral muscles of hens and ducks form unusually large quantities of lactic acid post-mortally. Heilbrunn (1928) inferred from proto-plasmic particles migrating to the cathode on passing an electic current in the cell, that some protoplasmic proteins are on the acid side of the isoelectric point. He further explained that an increase in the pH or alkalization of the protoplasm could affect some of the proteins and thereby bring about the release of the bound lipid.

It seems possible that there is a lipo-protein binding on either side, the acid as well as the alkaline side of the isoelectric point and changes in the pII could bring about the release of the lipid.   In the case of the fowl muscle the lactic acid formed on stimulation perhaps helps in the release of bound lipid which is also utilized.

The liver also contains significant quantities of lipid.   On electrically stimulating the breast muscle, the liver lipid also gets reduced.   Since the actual reduction in total lipid is more or less same as in free lipid, it is evident that no bound lipid was released from the liver on stimulation of the breast or leg muscle even in the fowl. That the liver is a depot for fat is well-known.   It is also known that this fat is utilized during the breeding period, especially in the female for depositing it in the eggs.   The present study has shown that liver fat may be utilized for locomotion also.

It is understandable that the leg muscle of the fowl takes 15 to 20 minutes to get fatigued, since some bound lipid is released and which seems to have been utilized.   Considering the large amount of free lipid present in the leg muscle of parakeet and bat, the time taken to get fatigued by these two muscles is relatively less than that taken by the leg muscle of the fowl.   The domestic fowl is a confirmed walker using its legs constantly, whereas the other two use their leg muscles in tonic contraction for perching.   As was stated in the case of the breast muscle of the kite, the mechanism for energy utilization in the leg muscle in tonic contraction while perching might be different from that in walking.

The general conclusion that can be drawn from this study is that the fuel in the flight muscles of birds and bats is to a great extent fat.   It is also brought out that when the fat supply in the muscle is depleted, that of the liver is drawn upon.   In the light of these studies, the long-distance flight of migrating birds like the golden plover becomes more easily understandable.   Weis-Fogh (1952) concluded that the long-range migrations of small flying animals could only be possible if fat were to be used as the fuel.   The present study supports the conclusions of Weis-Fogh.

The mechanism of fat utilization in the muscle remains to be explained.   The mitochondria from the mammalian liver cells are known to oxidize fatty acids (Kennedy and Lehninger, 1948).   It is most likely that the oxidation of fat takes place in the mitochondria of the muscle cells.   The fatty acids are converted into acetic acid and on further oxidation converted into carbon dioxide and water through the citric acid cycle, thus liberating energy.   With the oxidation of acetic acid, ketone bodies may be formed.   Increase in ketone bodies in the blood should also denote fat utilization, and that aspect is being investigated.

Summary

1.   There is a reduction in the free lipid of the breast and leg muscle as well as of the liver as a result of continuous excitation of the respective muscle in all the animals experimented with.

2.   In the case of the fowl alone there is any indication of utilization of bound lipid both in the breast and leg muscle as a result of continued excitation.

3.   The high percentage of lipids in the leg muscle of the domestic fowl indicates that it is an animal adapted for vigorous use of its legs.

4.   The reduction in liver fat as a result of prolonged stimulation of the breast muscle suggests the transport of fat from liver to the flight muscle.   It means that the liver serves as depot for fat to be utilized in any emergency.   Since muscle and liver both contain glycogen and fat as reserve fuel, they are complimentary tissues.

5.   Since the breast muscle of the kite gets fatigued sooner than that of the other animals experimented with, it appears to be unsuited for sustained flapping flight.   The soaring habit of the kite reflects this.

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THE RATE OF OXYGEN CONSUMPTION BY LIVE FISH PACKED IN PLASTIC BAGS

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T HE transport of live fish over long distances is often necessary to meet pisci-cultural requirements or to fulfil the demands of aquarists. Fish being easily perishable, transport was generally effected by air. This method has proved expensive in view of the freight to be paid on the containers with the water. Several methods have now been devised to pack live fish in reduced quantity of water. The method found most economical is the use of plastic bags. This method is comparatively simple and involves the use of compressed oxygen gas and a small quantity of water. The air in the bag above the surface of the water is expelled by constriction. A nipple connected with an oxygen gas cylinder is inserted into the compressed part of the bag until the entire space previously occupied by air is filled with pure oxygen gas. This gas keeps the oxygen content of water in such proportion as to meet the requirements of fish in the confined space during transit.

This method has successfully been employed for the transport to England of several consignments of live tropical fish by the Department of Fisheries. Bombay, even before announcement of the method in the issue of the " Aquarium Journal " for August 1953.

The method is being used on a large scale, but no data have, so far, been published on the rate of consumption of oxygen in the bags and the reason for the satisfactory survival of fish by this method. Some experiments in this direction were, therefore, undertaken.

Method

Bags of polythene plastic of 0·0025″ thickness and 33″ length and 24″ in width were used in the experiment. Each bag was introduced in a cardboard carton. measuring 17″ × 11″ × 11″, so that after it was filled with 10 litres of water. it assumed a uniform shape leaving equal space in the bag for oxygen up to a mark which was indicated on each bag at a uniform level. The pressure at which oxygen was filled in the bags from a gas cylinder was uniform at 60 lb. per sq. inch. which inside the bag became rarified to a pressure of about 4 lb. per sq. inch.

Experiment No. I : A total of 10 litres of water, containing 6 parts per million (p.p.m.) of dissolved oxygen, were introduced in each of two bags. In one bag,

125 live *Tilapia* sp. (100 of 1″ length and 25 of 1½″ to 2″ length) were introduced. The other bag, without any fish, was used as control. The bags were then filled with oxygen up to the mark, after expelling air. The condition of the fish was easily seen through the transparent texture of the plastic. After 48 hours, samples of water from each of the two plastic bags were estimated for oxygen content.

Experiment II : Same as in experiment I, except that the number of fish was increased from 125 to 250 (200 of 1″ length and 50 of 1½″ to 2″ length). After 48 hours, samples of water from each of the two bags were analysed.

Experiment No. III : Same as in experiment II, except that the two bags, instead of oxygen, were filled with atmopsheric air. After 48 hours, samples of water from each of the two bags were tested for oxygen content.

Results

The results of the experiments are summarised in Table I.

TABLE I

Parts per million (p.p.m.) of dissolved oxygen after 48 hours

Experiment No.	Water sample taken from		P.P.M. of dissolved 02. in the water	Temperature of water in Farenheit	Condition of fish
I. Bags filled with Oxygen gas	(a) Bag with 125 fish		9.9	80.0	Fish were comfortable. They were happily moving about.
	(b) Bag without fish		13.14	80.0	
II. Bags filled with Oxygen gas	(a) Bag with 250 fish		7.8	80.1	Fish were in good condition.
	(b) Bag without fish		13.15	80.0	
III. Bags filled with atmospheric air.	(a) Bag with 250 fish		3.1	80.2	Fish were somewhat in distressed condition. They were gasping for breath.
	(b) Bag without fish		8.2	80.2	

Discussion

The temperature of water inside the six bags after 48 hours, varied between 80·0° and 80·2° F., thus showing a maximum difference of only 0·2 degrees. It would not be incorrect, therefore, to assume that the dissolution of oxygen in the bag took place at a uniform temperature of 80·0°F.

Water containing oxygen initially at 6·1 p.p.m. showed an increase by only 2·1 p.p.m., when it remained in contact with atmospheric air for 48 hours (Experiment IIIb). Within the same period the oxygen content in water in experiments

I(b) and II(b) increased from 6·1 to 13·14 and 13·15 p.p.m. respectively, when it remained in contact with pure oxygen gas for 48 hours. The pure oxygen gas thus provided a maximum of 13·1 p.p.m. of dissolved oxygen i.e. an increase by 7 p.p.m. as compared to an increase of only 2·1 p.p.m. when water was in contact with air. About 5 p.p.m. more oxygen was dissolved in water when it was in contact with pure oxygen than with atmospheric air.

The water in the three bags with fish in experiment I(a), II(a) and III(a) had at the end of 48 hours oxygen contents of 9·9, 7·8 and 3·1 p.p.m. respectively. It was observed that when the oxygen content of water was lowered to 3·1 p.p.m., fish were in a distressed condition, although none died. It may, therefore, not be quite incorrect to conclude, that if oxygen content was reduced to 3 p.p.m. fish may not have lived longer than a few more hours in the plastic bags. It was observed that by introducing 125 more fish as in experiment II(a), the oxygen content of water was reduced from 9·9 p.p.m., to 7·8 p.p.m. i.e. 2·1 p.p.m.

Considering that less than 3 p.p.m. of oxygen in water is a danger limit and 125 fish (25 of $1\frac{1}{2}$″ to 2″, and 100 of 1″ size) bring about a reduction of 2· 1 p.p.m. after an interval of 48 hours, it can be concluded that 250 fish (200 of 1 inch and 50 of $1\frac{1}{2}$″ to 2″ size), can be safely transported over distances involving 96 hours' journey.
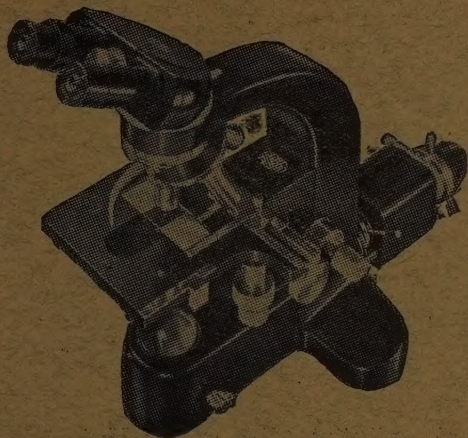
Summary

1.   Experiments involving confining of fish in ploythene plastic bags for 48 hours under varying oxygen contents were conducted.

2.   In bags containing 125 fish, with oxygen content of 9·9 p.p.m., the fish were found to be comfortable.

3.   In bags containing 250 fish and oxygen, content of 7·8 p.p.m., the fish were found to be in good condition.

4.   In bags containing 250 fish and oxygen content of 3·3 p.p.m., the fish were found to be in a distressed condition gasping for breath.

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